# A Systems Approach to the COP9 Signalosome[w]

The COP9 signalosome (CSN) was identified close to a decade ago (Wei et al., 1994). The biochemical purification of the CSN (Chamovitz et al., 1996) was a major breakthrough, because it showed that Arabidopsis genetics could be approached biochemically, and it subsequently lead to the cloning of all CSN subunits based on the peptides from the purified subunits (Kwok et al., 1998; Karniol et al., 1999; Serino et al., 1999, 2003; Peng et al., 2001a, 2001b). As predicted, most of the CSN subunits are encoded by the pleiotropic *cop/det/fus* loci. Although originally described as a master repressor of photomorphogenesis in plants, subsequent studies have shown that the CSN also regulates multifaceted developmental processes in plants (Peng et al., 2001a, 2001b); a specific role in light signaling has also been established (Kang et al., 2000; Ma et al., 2003). The study of the CSN is a clear example of Arabidopsis research leading the way for animal research, because it is now clear that the CSN also has multiple essential roles in non-plant systems (Chamovitz and Deng, 1995; Oron et al., 2002).

Biochemically, the CSN has been implicated in two distinct processes: regulation of protein degradation through deneddylation of the cullin subunit of multiple SCF (Skpl/cullin/F-box) E3-ubiquitin ligases and modulation of kinase signaling pathways through associated kinases (for review, see Schwechheimer and Deng, 2001; Bech-Otschir et al., 2002). In addition, roles in regulating the proteasome and eIF3 have been proposed (for review, see Kim et al., 2001). With the recent advent of "systems biology" approaches (Kitano, 2002), the CSN is poised once again to be at the forefront of a research paradigm shift.

The classification of the *cop/det/fus* loci as pleiotropic underscores the need for a systems approach to studying the CSN. Although we can continue to look at individual subunits or at isolated developmental processes, one has to question the efficiency of these studies in understanding the developmental function of the CSN. Considering that it is now clear that CSN subunits are found simultaneously in multiple configurations, that mutations in one subunit are known to affect the configuration of other subunits, that multiple proteins interact with the CSN, and that the CSN effects multiple pathways, it is not clear that a reductionist approach can adequately explain CSN function in development. Despite the accumulating data, central questions remain: Which pathways does the CSN regulate? How does the CSN simultaneously regulate multiple pathways through diverse mechanisms? Where does the "activity" lie—in the complex or in individual subunits? Do individual subunits have unique roles independent of the complex or within the complex? On a more global level, is the CSN circuitry conserved in animals? Can we identify distinct shared or unique nodes that impinge on or are regulated by the CSN?

Although "systems biology" has grand overtones, we prefer to look at it as a return to physiology on a larger scale, an "integrative physiology." What characterizes this approach is that rather than studying individual components of a system, as has been the reductionist paradigm for the past 30 years, it looks at all the components of a system simultaneously and in conjunction with each other (Ideker et al., 2001). Although ultimately a systems approach hopes to encompass every gene and protein for a given developmental process (e.g. Davidson et al., 2002), there is still room for a reductionist approach in the global study of key nodes. A systems approach predicts key nodes in the network, which have profound effects. The CSN may be one of these nodes.

Is CSN research ripe for a systems approach? For this, we need to identify all components of the complex, the proteins that interact with the complex (direct targets) and proteins that further interact with the direct targets (downstream targets), generate multiple mutations or perturbations in all components of the system, and generate comprehensive sets of quantitative data, including DNA and protein expression profiles. A more complete data set would also contain information on component phosphorylation status, etc. This data should be quantitative over temporal and spatial scales.

We are now at the stage where the amount of data accumulating could allow such an approach. Interacting proteins have been reported for a number of subunits, and exhaustive interaction-trap screens are being carried out in several labs, which can serve as a base for the development of an interaction network. Mutants or transgenic hypomorphs have been reported for all plant CSN subunits, and the available mutant collections can easily provide mutants for most interacting proteins. Expression data covering hundreds of conditions are available for most of these components as they are included in the public Arabidopsis microarray data (ftp://tairpub:tairpub@ftp.Arabidopsis.org/home/tair/Microarrays/). Initial microarray experiments on two of the CSN mutants were recently published (Ma et al., 2003) and more can be expected. Our lab is working on developing a proteomic description for CSN mutants. Per-

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haps surprisingly, two-dimensional profiles of mutants in the CSN are not too different from the wild type (see supplemental Fig., available at http://www.plantphysiol.org), which suggests that we may need to look for qualitative differences, such as differences in phosphorylation status, rather than changes in global patterns.

Because the CSN is not a direct regulator of transcription, it may be asked what transcript profiling will provide. The CSN does, however, regulate key transcription factors such as HY5 (Schwechheimer et al., 2002), so profiling juxtaposed with cis-regulatory analysis could be invaluable in identifying many of the pathways regulated by the CSN. A similar approach has been demonstrated in studying sea urchin embryogenesis (Davidson et al., 2002). Thus one research model would entail identification of all transcription factors regulated by the CSN, generation of mutants, and the comparison of transcript profiles of mutants for these factors with profiles from CSN mutants to determine which subset of the *cop* phenotype can be explained through a particular pathway.

What differentiates a systems approach from a simple brute force/cataloging approach is the integration of computer science. This is not bioinformatics in its most widely used contexts today, but it is rather the use of algorithms and model building to develop new hypotheses and to test them before deciding on the experimental direction. Thus computers are used simultaneously for organizing experimental data and as active research tools at each stage of an experimental program. Such an approach can help put quantitation into developmental biology and can help to reemphasize the use of hypothesis-driven biology in our research. Such modeling should help us in defining our research question, such as: Are we modeling light signaling with the CSN as a component? Or are we modeling the CSN with light signaling as an output? One other advantage of this approach is that because much of the data is public, modeling and hypothesis generating are not limited to the large well-funded labs. Perhaps the greatest strength in this multifaceted approach is that we can approach our experimental system a priori with little prejudice, allowing us to make sense of the pleiotropy inherent in the CSN and so many other key regulators.

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